Neural Re-adaptation to Earth's Gravity Following Return from Space

Authors

Richard Boyle, Allen F. Mensinger, Kaoru Yoshida, Shiro Usui, Anthony Intravaia, Timothy Tricas, Stephen M. Highstein

Experiment Team

Principal Investigator: **Stephen M. Highstein**[1]

Co-Investigators: **Richard Boyle,**[2,5] **Allen F. Mensinger,**[1] **Kaoru Yoshida,**[3] **Shiro Usui,**[4] **Timothy Tricas**[6]

Engineer: **Anthony Intravaia**[5]

[1]Washington University School of Medicine, St. Louis, USA
[2]Oregon Health Sciences University, Portland, USA
[3]University of Tskuba, Tskuba, Japan
[4]Toyohashi University of Technology, Toyohashi, Japan
[5]NASA, Ames Research Center, Moffett Field, USA
[6]Florida Institute of Technology, Melbourne, USA

ABSTRACT

The gravity sensors in the inner ear (the utricle and saccule) no longer receive strong gravitational signals in weightlessness. In an effort to compensate for the reduced input, these sensors may become more sensitive using a process called up-regulation. If this happens, the output of the vestibular nerve from the inner ear would be greatly increased after a space mission, when the sensitized gravity sensors are reexposed to gravity. We believe that information gathered by studying the inner ear of a lower animal, such as a fish, will provide the information needed to prove this. Despite evolution, the balance and equilibrium functions of the inner ear have not appreciably changed since their appearance in the earliest vertebrates. As a result, the fish vestibular system compares favorably in both structure and function with that of mammals. By chronically recording the output of the vestibular system in the fish (specifically the output from the utricle), the question of how microgravity affects the output of the inner ear could be answered precisely. For five days following two NASA Shuttle flights, we recorded from the vestibular nerves supplying the utricle the responses to inertial accelerations (head movements) in four oyster toadfish (*Opsanus tau).* Within the first day postflight, the magnitude of response to an applied translation (side-to-side) movement was on average three times greater than for controls. The reduced gravitational acceleration in orbit apparently resulted in an up-regulation of the sensitivity of the utricle. By 30 hours postflight, responses were statistically similar to control. The time course of return to normal sensitivity parallels the reported decrease in vestibular disorientation and improvement in balance in astronauts following their return from space.

INTRODUCTION

It is fundamentally important that organisms remain orientated within their terrestrial environment. Vertebrates possess a gravity-sensing system, the utricular and saccular organs, that senses the sum of forces due to head movements and transforms the sum of these accelerations into a neural code. This code is combined with acceleration signals from the semicircular canals and with information derived from other sensory modalities (such as vision and position sense) to compute a central nervous system representation of the body in space that is called the gravitoinertial vector. In this way, the central nervous system resolves the ambiguity of signals due to gravity and to self-motion, thereby maintaining balance and equilibrium under varying conditions.

Exposure to microgravity imposes an extreme condition to which the traveler must adapt. Many, if not most, human travelers experience some disorientation and motion sickness during the first few days in microgravity. This effect is called the space adaptation syndrome. This syndrome is akin to terrestrial motion sickness (Reason, 1975). From studies on the earliest space crews, it was evident that adjustments to the microgravity environment occur in flight and then reverse upon return to Earth's gravity (Black, 1999). The specific adaptation mechanisms are conjectural and could range from neural to structural changes or both. We studied the neural re-adaptation to Earth's gravity using electrophysiological techniques to measure the nerve signals from the inner ear (utricular nerve afferents) in fish upon return from exposure to microgravity.

METHODS

Six oyster toadfish (*Opsanus tau*), weighing 150–700 gm, were individually housed in seawater tanks aboard two NASA Shuttle missions (Neurolab and STS-95). The fish were returned to the laboratory where all experiments were performed within ~10 hours of the Shuttle landing. Surgical procedures, similar to those described by Boyle and Highstein (Boyle, 1990), were performed in accordance with the American Physiological Society Animal Care Guidelines and approved by the international Animal Care and Use Committee. Fish were anesthetized with MS-222 (Sigma) and secured in a Plexiglas tank placed atop an experimental table. The utricle and its afferent nerve were exposed, and extracellular potentials were recorded from individual nerves. The experimental apparatus allowed for a variety of accelerations and movements to be applied to the fish (manual yaw rotation about Earth vertical, translational acceleration parallel to Earth horizon, and/or static tilt with respect to gravity). The fish could be repositioned in a 360-degree circle such that: (a) the translational acceleration was delivered along any direction in the horizontal head plane, and (b) the acceleration was specifically directed; e.g., nose-down (pitch) or side-down (roll). Static sensitivity of otolith afferents to gravity was observed both in control and in postflight fish.

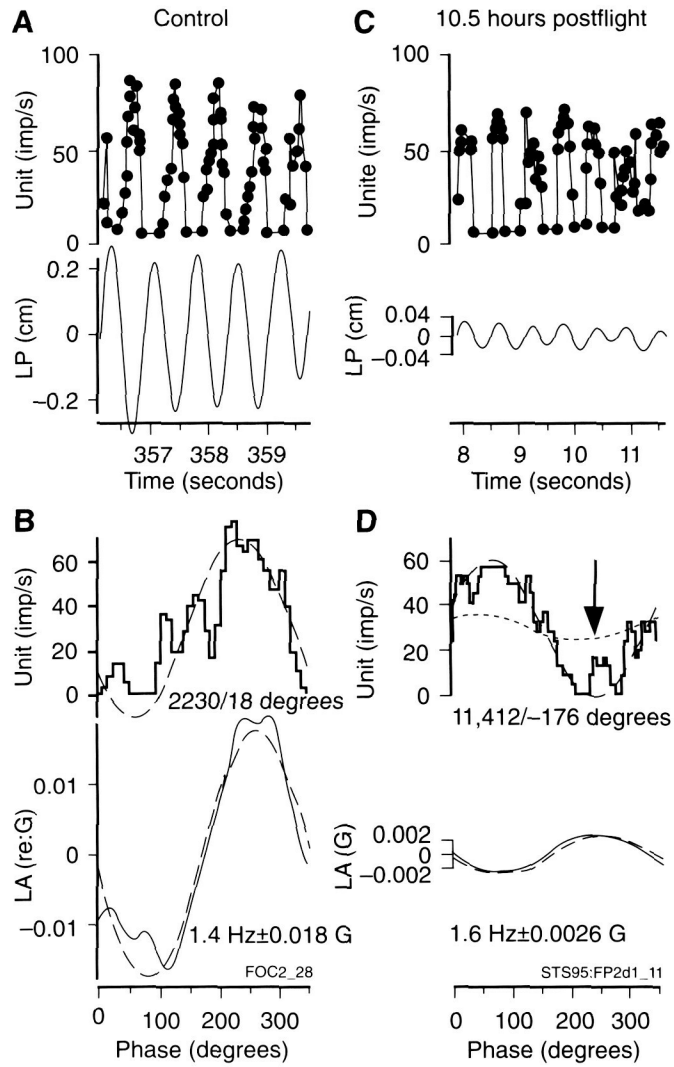

Figure 1. Control (A, B) and 10.5-hour postflight of STS-95 Shuttle (C, D) responses of oyster toadfish utricular afferents to translational (side-to-side) accelerations. The nerves recorded shortly after return to Earth exhibit a profound hypersensitivity to translational accelerations. The amplitude of the applied 1.6-Hz stimulus in C and D was almost negligible (±0.0026 G) but induced a firing rate modulation of ~±30 impulses/second (or 11,412 impulses/second/G). An average control afferent would exhibit only ~±4 impulses/second modulation for this weak stimulus. The control response of the preflight afferent (shown in A and B) is modeled as the dashed curve marked by the arrowhead in the histogram of the postflight afferent in D.

A, C: Afferent firing rate (in impulses/second; upper trace) is sinusoidally modulated to an applied sinusoidal change in linear position ((LP), in cm; lower trace). In both records, the fish were rotated counterclockwise by 90 degrees about the vertical axis, resulting in a maximum rate increase for an acceleration directed rightward along the inter-labyrinth axis, on the same side as the recorded control afferent in A and in the opposite sense for the postflight afferent in C.

B, D: Averaged response (upper histogram) of records shown in A and C. Lower trace shows the averaged linear acceleration ((LA) re:G) of each stimulus. Ordinates in each panel are scaled equally to illustrate the primary finding. (From Boyle, 2001, with permission, reproduced from the *Journal of Neurophysiology*.)

RESULTS

Four toadfish were flown on the STS-90 Neurolab mission (16 days), and two toadfish were flown aboard the STS-95 mission (nine days). Two fish survived Neurolab, and both fish survived STS-95. Responses of utricular nerves to gravitational (tilt) and inertial (translation) accelerations were recorded from four flight fish.

Control responses were obtained from 32 utricular nerves in three fish. Figure 1 shows the firing rate response (upper trace, in impulses/second) to a sinusoidal change in linear position ((LP), lower trace). In Figure 1A, the fish was first rotated about the vertical axis in a counterclockwise step to a 90-degree head (and body) angle. The resulting linear acceleration ((LA), Figure 1B) was directed to the right on the same side as the recorded afferent along the inter-labyrinth axis. This maximally excited the afferent. The averaged response to five stimulus cycles had a maximal sensitivity of 2230 impulses/second/G (Figure 1B; see Table 1).

Hair cell bundles are morphologically polarized, and hair cell receptor potentials are directionally sensitive to bundle displacement. Directional selectivity of utricular nerves is distributed in a fanlike shape (Fernández, 1976), as expected from hair cell orientations in the utricular macula (Spoendlin, 1966). Figure 2 shows the test used to determine the directional selectivity of individual afferents in control (A) and postflight (B) fish. A sinusoidal translational acceleration along an Earth-parallel plane was delivered at successive 15 degree positions after the fish was stepped around a 360-degree circle. Head angle (degree) was defined using a right-hand rule relative to the laboratory: a positive acceleration at zero degree represents a forward movement directed out the fish's mouth, and one at 90 degrees represents a movement directed out the fish's right ear. Directional selectivity was determined by plotting the response sensitivity and phase relative to head angle. The data were fit by a rectified cosine function (dashed lines in A and B); and correspondence between the tested and predicted responses reflects the sharpness of directional tuning. The control nerve in A was sharply tuned and directionally selective to acceleration directed along the inter-labyrinth axis. All control nerves were directionally selective, and the maximal response vectors spanned 360 degrees of head angle.

In early postflight fish, utricular nerves were hypersensitive to translational acceleration (Figures 1-3) and directionally selective (Figure 2B). One of the first nerve fibers recorded at 10.5 hours postflight illustrates the striking increase in response sensitivity when stimulated at ±0.0026-G acceleration or ±0.025-cm displacement (Figures 1C, 1D). The discharge modulation was ~±30 impulses/second, yielding a maximal sensitivity of 11,412 impulses/second/G, nearly seven-fold greater than the control mean and ~three-fold greater than the maximum response obtained in any individual control (4136 impulses/second/g; Table 1).

Data for both STS-90 and STS-95 Shuttle missions are presented in the form of a probability plot (Figure 3) to show the initial increase and recovery of response sensitivity. The data in this figure and in Table 1 are divided into groups based on time postflight. Maximum sensitivity of each nerve is plotted as a

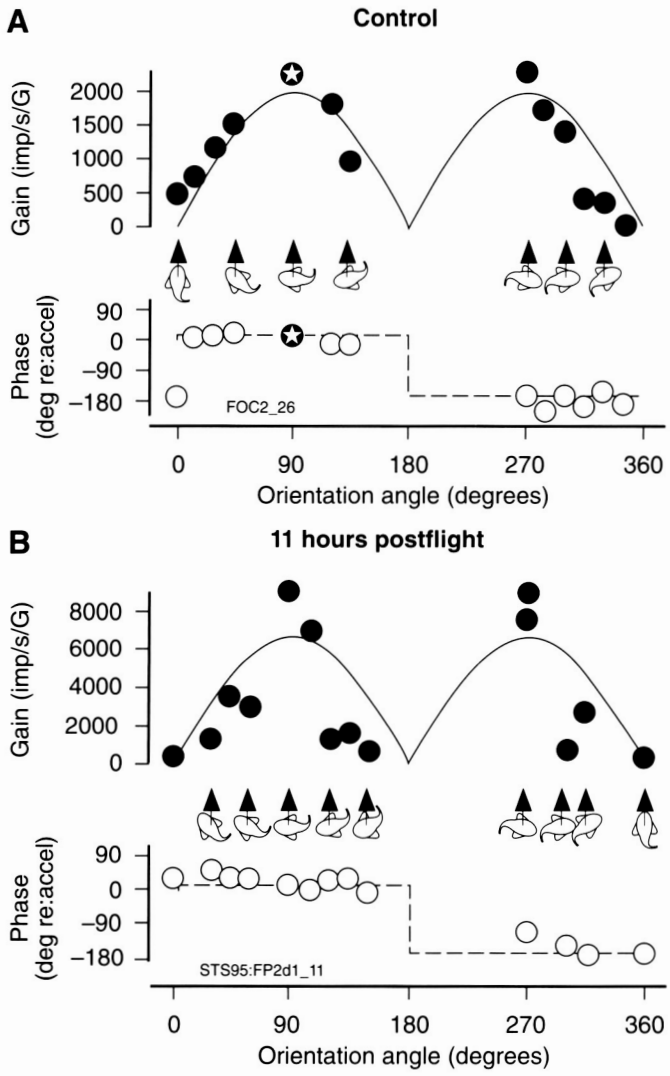

Figure 2. Directional selectivity of control (A) and 11 hours postflight of STS-95 Shuttle (B) toadfish utricular afferents to translational (side-to-side) accelerations. A, B: Sensitivity (solid symbols) and phase (open symbols) are plotted as a function of orientation angle (in degrees) of the animal. The small fish drawn between the sensitivity and phase plots graphically depict the orientation as viewed from above for selected tests; by convention, forward motion of the test sled is denoted as an arrowhead. The afferents recorded exhibited an enhanced sensitivity to applied linear accelerations and directional selectivity. (Note the difference in scale for gain between the control and the 11-hour-postflight graphs.)

The individual response indicated by the star in panel A represents the response shown in Figure 1A, B. Forward acceleration out the fish's mouth is given as zero degree, and the fish is rotated counterclockwise about the Earth's vertical axis and the designated angles follow a right-hand rule. For example, 90 degrees is an acceleration directed rightward along the inter-labyrinth axis, 180 degrees is a backward acceleration out the fish's tail, and 270 degrees is an acceleration directed leftward along the inter-labyrinth axis. The response of utricular afferents follows a rectified cosine function (dashed curves fit to the empirical data) with respect to orientation angle, indicating directional selectivity. A: Control afferent. B: afferent recorded 11 hours postflight of STS-95 Shuttle. (From Boyle, 2001, with permission, reproduced from the *Journal of Neurophysiology*.)

Table 1. Summary of control and postflight utricular afferent data.

Fish	Hours Postlanding	Mean Max. Sensitivity (impulses/second/G)
3 Controls		168 ± 1195 (61–4136; n=32)
STS-95: 1+2	10–16	3772 ± 3607* (142–13290; n=24)
STS-95: 2	29.5–32	1582 ± 1750 (101–4992; n=16)
STS-95: 2	52–55	1195 ± 1478 (126–5861; n=18)
STS-90: 2	53–59	1399 ± 1599 (116–7819; n=29)
STS-95: 1+2	70–76	1337 ± 1076 (100–4738; n=30)
STS-90: 3	112–117	1476 ± 951 (154–3685; n=28)

*$p<0.01$

Comparison of maximum response sensitivity (impulses/second/G) of utricular afferents recorded under control conditions to those at different times postflight of STS-90 (two fish labeled 2 and 3) and STS-95 (two fish labeled 1 and 2). At 10-16 and 70-76 hours postflight, the results obtained from the two STS-95 fish were comparable and are combined. The number of afferents at 10-16 hours is eight (STS-95: 1) and 16 (STS-95: 2). At 70-76 hours, the number of afferents is 10 (STS-95: 1) and 20 (STS-95: 2). Mean and one standard deviation, with range of smallest to largest and number (n), are given in parentheses. Results show that the sensitivity was significantly greater than control ($p<0.01$) for the earliest recording session (10–16 hours) from the two fish flown on STS-95. Results obtained from flight fish at later periods were not significantly different from control. The first records taken from fish flown on STS-90 began 53 hours postflight after re-adaptation to Earth's gravity.

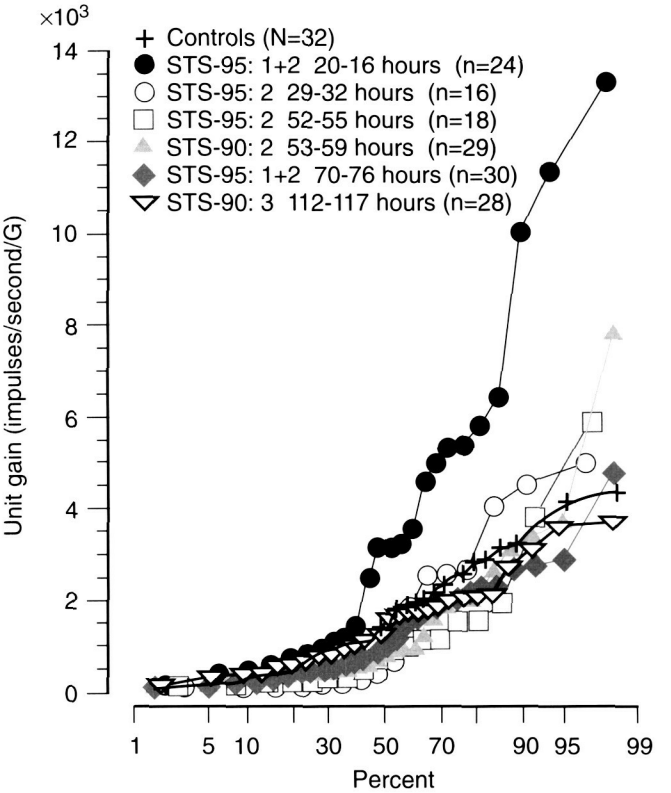

Figure 3. Hypersensitivity of toadfish utricular afferents to translational (side-to-side) accelerations within the first day postflight of STS-90 and STS-95. Groups are formed with respect to the time postflight, from the earliest time of 10–16 hours for both fish (1+2) of STS-95 (filled circles) to the latest time of 112–117 hours for fish labeled 3 of STS-90 (inverted open triangle). Maximum sensitivity of each nerve is plotted as a percentage of the population, with sensitivity less than that of the measured nerve. For example, for roughly 60% of the nerves (14/24) in both fish on STS-95 (filled red circles), labeled STS-95: 1+2, the sensitivity recorded 10–16 hours postflight was dramatically enhanced relative to control (crosses). (From Boyle, 2001, with permission, reproduced from the *Journal of Neurophysiology*.)

percentage of population sensitivity with a value less than the individual sensitivity. For roughly 60% of the nerves (14/24) in both fish on STS-95 (filled red circles), labeled STS-95: 1+2, the sensitivity recorded 10–16 hours postflight was dramatically enhanced relative to control (crosses). Within this time group, the sensitivity of the entire sample (n=24) was roughly triple that of the control ($p<0.01$). The sensitivity returns to near normal at the recorded time of 29.5–32 hours postflight and remains within normal range after five days postflight.

To examine for possible recording bias, all measured parameters were compared between control and postflight nerves. No statistical difference was found in the range and mean of discharge rate (impulses/second) and regularity of discharge (standard deviation of the interval divided by the mean interspike interval) between postflight and control fish. An equal distribution of head angles was also found, evoking maximum and minimum response modulations postflight, similar to those observed in control fish, and response phase (degree).

DISCUSSION

The increase in sensitivity of certain inner ear nerves following space travel is most likely due to the animal being exposed to microgravity. The results, however, should be regarded as preliminary because single nerve fibers were not studied sequentially. The findings could be explained by changes at a number of locations within the inner ear (see illustration at the beginning of this section for a description of the anatomy). Possible explanations include: (1) an increase in the sensitivity of the hair cells, (2) a temporary structural alteration affecting the ability to convert otolith movement of the stones into nerve impulses, (3) a change in coupling between the otolith and hair cells, causing enhanced deflection of hair cell bundles for a given movement, or (4) an alteration in the strength of synaptic transmission. The number of synaptic ribbons in certain Type II

hair cells in rodents is labile, increasing after exposure to microgravity (Ross, 2000) (see science report by Ross et al. in this publication). Toadfish possess only Type II hair cells. The number of synaptic boutons on these cells and the response sensitivity to vestibular stimulation are correlated (Boyle, 1990). Thus, an increase in number of synaptic ribbons in toadfish otolith hair cells following exposure to microgravity could potentially explain the present results.

If the inner ear structure is arranged for optimal responses in one-G, mechanical alterations may also occur in microgravity. For example, loss of gravitational force might displace the otolithic membrane relative to the macula, thus affecting neural responses. Altered gravity conditions might also trigger an adaptive response of the weight-lending structures (Weiderhold, 1997) (see science report by Weiderhold et al. in this publication). It is clear that more experiments under controlled states of altered gravity are required to determine the structural and developmental response of the inner ear and the consequence of spaceflight on inner ear function.

Adaptation of hair cell receptor potentials occurs to prolonged deviation of their sensory hair bundle (Eatock, 1987). Unweighting of the otolith mass in microgravity might cause an adaptation of receptor potentials. That the enhanced sensitivity remained for at least 24 hours, substantially longer than the suggested time course of adaptation, is inconsistent with this view.

Otolith sensors provide a major input to the internal representation of the gravitoinertial vector. Thus, an abnormal utricular or saccular component should have profound effects upon the orientation of the organism, and has been hypothesized to be causal in vestibular disorientation or space adaptation syndrome. The demonstrated time course in the altered responses parallels the time course of disorientation experienced by space travelers, and gives support to this hypothesis.

The earliest recordings began 10 hours after STS-95 landing. To what extent this delay affects the interpretation of the data is indeterminate. Because of enhanced afferent sensitivity, the initial postflight results were limited to fewer stimulus frequencies (1–2 Hz) and to lower amplitudes than were delivered in control tests. These restrictions in stimulus parameters were required to minimize discharge nonlinearity (Boyle, 1990). Sensitivity on average declined from day two to day four, and larger stimulus amplitudes could be progressively applied. The first single-unit recordings after the STS-90 flight began 53 hours after landing, well after the postflight recovery time observed in the STS-95 fish. Significantly, the directional tuning of nerves remained unchanged after exposure to microgravity. We therefore would not expect to find that significant remodeling of the spatial extent of dendritic arbors within the sensory epithelium has occurred.

To date, the toadfish utricular nerve has only been crudely evaluated at one frequency with a hand-powered linear sled. If the fish utricle bears any resemblance to similar epithelia studied in other species, more complete functional evaluations of afferents will no doubt demonstrate considerable diversity. It therefore remains to be tested whether a specific population demonstrated increased sensitivity or whether this finding is a general feature of all cells.

Acknowledgements

This work was supported by grants from NASA Ames # 2-945 and NIH/NIDCD PO-1 DC 01837 (USA) and NASDA (Japan).

REFERENCES

THE VESTIBULAR SYSTEM AND ITS DISEASES. H.H. Spoendlin. edited by R.J. Wolfson. Philadelphia: University of Pennsylvania Press, pages 39–68; 1966.

MOTION SICKNESS. J.T. Reason and J.J. Brand. Academic Press, London; 1975.

PHYSIOLOGY OF PERIPHERAL NEURONS INNERVATING OTOLITH ORGANS OF THE SQUIRREL MONKEY. II. Directional selectivity and force-response relations. C. Fernández and J.M. Goldberg. *J. Neurophysiol.*, Vol. 39, pages 985–995; 1976.

ADAPTATION OF MECHANOELECTRICAL TRANSDUCTION IN HAIR CELLS OF THE BULLFROG'S SACCULUS. R.A. Eatock, D.P. Corey, and A.J. Hudspeth. *J. Neurosci.*, Vol. 7, pages 282–236; 1987.

RESTING DISCHARGE AND RESPONSE DYNAMICS OF HORIZONTAL SEMICIRCULAR AFFERENTS OF THE TOADFISH, *OPSANUS TAU. R.* Boyle and S.M. Highstein. *J. Neurosci.*, Vol. 10, pages 1557-1569; 1990.

DEVELOPMENT OF GRAVITY-SENSING ORGANS IN ALTERED GRAVITY CONDITIONS: OPPOSITE CONCLUSIONS FROM AN AMPHIBIAN AND A MOLLUSCAN PREPARATION. M.L. Wiederhold, H.A. Pedrozo, HJ.l. Harrison, R. Hejl, and W. Gao. *J. Grav. Physiol.*, Vol. 4, pages P51–P54; 1997.

DISRUPTION OF POSTURAL READAPTATION BY INERTIAL STIMULI FOLLOWING SPACE FLIGHT. F.O. Black, W.H. Paloski, M.F. Reschke, M. Igarashi, F. Guedry, and D.J. Anderson. *J. Vestib. Res.*, Vol. 9, pages 369–78; 1999.

CHANGES IN RIBBON SYNAPSES AND ROUGH ENDOPLASMIC RETICULUM OF RAT UTRICULAR MACULAR HAIR CELLS IN WEIGHTLESSNESS. M.D. Ross. *Acta Otolaryngol.*, Vol. 120, pages 490–499; 2000.

NEURAL READAPTATION TO EARTH'S GRAVITY FOLLOWING RETURN FROM SPACE. R. Boyle, A.F. Mensinger, K. Yoshida, S. Usui, A. Intravaia, T. Tricas, and S.M. Highstein. *J. Neurophysiol.*, Vol. 86, pages 2118-2122; 2001.